NOTES

Membrane Vesiculation Function and Exocytosis of Wild-Type and Mutant Matrix Proteins of Vesicular Stomatitis Virus

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Transfection of mammalian CV1 cells with a recombinant M-gene pTM1 plasmid, driven by vaccinia virus-expressed phage T7 polymerase, resulted in the expression of matrix (M) protein, which is progressively released from the exterior surface of the transfected-cell plasma membrane. Exocytosis of M protein begins 2 to 4 h posttransfection and reaches a peak by 10 to 16 h posttransfection; dye uptake studies reveal that >97% of cells are alive and have intact membranes at 16 h posttransfection. Density gradient centrifugation and labeling with radioactive palmitic acid revealed that the M protein is released from cells in association with lipid vesicles. Expression of M-gene deletion mutants suggests that exocytosis of M protein requires the presence of a membrane-binding site at N-terminal amino acids 1 to 50. Cells transfected with the pTM1 plasmid containing the M gene of the temperature-sensitive mutant *tsO23* expressed ample quantities of the mutant M protein at permissive (31°C) and restrictive (39°C) temperatures, but the exocytosis of the mutant M protein occurred only at the permissive temperature. The *tsO23* M gene has three site-specific mutations resulting in amino acid substitutions at residues 21, 111, and 227. Expression of wild-type and mutant M genes with mutations or revertants at each of these sites resulted in exocytosis of M protein at the nonpermissive temperature only when wild-type leucine was present at residue 111, but M-protein exocytosis was restricted (to some extent even at the permissive temperature) when mutant phenylalanine was present at residue 111. Past and present data indicate that a specific structural conformation of the M protein is responsible for the formation and budding of vesicles, a property of the M protein which probably also promotes vesicular stomatitis virus assembly and budding of virions from host cells.

Vesicle formation from the plasma membrane of mammalian cells has been studied primarily by endocytosis of clathrin-coated pits that invaginate and bud into the cytoplasm (2). Although protein-induced vesiculation and exocytotic budding have been studied, it has not been in any detail and then largely by means of retrovirus Gag proteins, such as the human immunodeficiency virus type 1 (HIV-1) precursor Pr55^{gag}. When expressed by itself, Pr55^{gag} assembles with and evaginates from the plasma membrane, leading to the formation and budding of lipoprotein vesicles resembling HIV virions (7, 15); however, it is unclear which of the Gag protein cleavage products induces membrane exocytosis. Very recent studies reveal that single-amino-acid changes in HIV-1 matrix protein also block virus particle production (6). A 25-kDa cleavage product of the Rous sarcoma virus Gag protein appears to be the component of this retrovirus that causes membrane evagination and release of vesicles (19). The 26-kDa matrix (M) protein of vesicular stomatitis virus (VSV), when expressed by a baculovirus vector, also has been shown to induce the formation and budding of vesicles from the external surface of insect cells (9). Here we report that transient expression of the VSV M protein alone induces formation of vesicles that are released from the

mammalian cell plasma membrane into the surrounding culture medium. This membrane-budding function is also shown to be restricted by a specific site mutation in the M-gene cDNA of a VSV temperature-sensitive mutant, in which phenylalanine is substituted for leucine at amino acid 111 of the M protein. This mutation previously had been shown to be responsible for restricted production of virions at a nonpermissive temperature (11), which can also be rescued by a coexpressed revertant (10).

The wild-type (wt) M gene studied here is a cDNA clone coding for all 229 amino acids of the VSV M protein; it was obtained from mRNA of VSV-Indiana (Orsay strain) and was cloned in this laboratory into a vector designated pYL-OM79, flanked by promoter and terminator sequences for bacteriophage T7 RNA polymerase (10). We had also created deletion mutants of the M gene by restriction enzyme cleavage or by PCR which gave rise to truncated proteins in which the first 50 amino acids were deleted or amino acids 75 to 229 were deleted, as well as a double-deletion mutant missing amino acids 1 to 50 and 75 to 106 (20). In addition, the cDNA of the M gene of the temperature-sensitive mutant *tsO23* had also been cloned in the same expression vector (10); the recombinant *tsO23* M gene gave rise to a protein with amino acid substitutions at residues 21, 111, and 227 (11). Also available from earlier studies were mutant clones of the wt M gene which gave rise to proteins with single-amino-acid substitutions at residues 21, 111, and 227 identical to those of the *tsO23* mutations, as

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well as revertants in the *tsO23* M gene expressing proteins with individual revertants at amino acids 21, 111, and 227 (10). To provide maximum expression, the pYL-OM79 wt M gene, its deletion mutants, the *tsO23* M gene, each single-site mutant M gene, and each single-site revertant M gene (as well as the VSV N, P, and G genes) were subcloned into the *Nco*I and *Eco*RI sites of the vaccinia virus-based, cap-independent pTM1 expression vector (12) (a gift of Bernard Moss).

Transient expression of proteins was carried out, as previously described (17), with certain modifications, by first infecting subconfluent CV1 cell monolayers for 30 min with the T7 polymerase-expressing vaccinia virus vTF1-6,2 (multiplicity of infection, 10 PFU per cell) and by subsequent transfection with 10 μ g of cDNA in the presence of 10 μ l of Lipofectin (GIBCO). After transfection for 2 h at 37°C, cells were washed and depleted of endogenous methionine by incubation in 1 ml of methionine-free minimal essential medium for 30 min and replaced with 1 ml of methionine-free minimal essential medium containing 100 μ Ci of [³⁵S]methionine. After incubation at 37°C (or at 31 and 39°C for *ts* mutant clones) for various times up to 16 h, the culture medium was harvested for analysis of released viral proteins. Cell culture medium fractions (900 μ l each) were clarified by centrifugation at 5,000 $\times$ g for 5 min and exposed to 100 μ l of 10 $\times$ NET gel buffer (0.5 M Tris-HCl [pH 7.5], 1.5 M NaCl, 1% Nonidet P-40, 10 mM EDTA, 2.5% gelatin, 0.2 M sodium azide). CV1 cell monolayers were washed twice with phosphate-buffered saline (PBS), lysed in 0.5 ml of radioimmunoprecipitation assay buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 2 μ g of aprotinin per ml), and clarified by centrifugation at 7,000 $\times$ g for 5 min. The clarified culture media and cell lysates were then incubated for at least 2 h at 4°C with the previously described monoclonal antibodies directed to the M protein (16), before absorption on protein A-Sepharose beads (Sigma) at 4°C for at least 1 h. The antigen-antibody complexes adhering to the protein A-Sepharose beads were collected by centrifugation, washed three times in 1 $\times$ NET gel buffer and once in STE buffer (0.1 M NaCl, 10 mM Tris-HCl [pH 8.0], 1 mM EDTA) before the addition of Laemmli buffer. The ³⁵S-labeled proteins were analyzed by electrophoresis on a 12.5% polyacrylamide-SDS gel and autoradiography.

Our initial aim was to determine whether the VSV M protein, expressed in the absence of the other VSV proteins, is released from intact mammalian cells. To this end the M protein and, as controls, other viral proteins were expressed individually in CV1 cells transfected with VSV cDNAs cloned in the plasmid pTM1 driven by the T7 phage polymerase expressed by the coinfecting vaccinia virus recombinant vTF1-6,2. Figure 1 shows that considerable amounts of [³⁵S]methionine-labeled M, N, P, and G proteins were present in the cytosol of transfected cells incubated at 37°C for 8 h. However, only the M protein and a truncated form of the G protein were released into the culture medium surrounding the intact cells (Fig. 1A). A soluble form of the G protein (designated G_s), devoid of about 50 carboxy-terminal amino acids, is formed by and secreted from infected cells by proteolytic cleavage or by premature termination of the G protein (8), which in our studies was released from cells in a lipid-free, faster-migrating form (Fig. 1A, lane 8). Cells coexpressing N and M proteins or P and M proteins released only the M protein (data not shown). Autoradiographic electropherograms showing the time course of M-protein synthesis and release reveal increasing cytoplasmic accumulation of M protein from 2 to 12 h posttransfection with pTM-O79; M protein released from transfected cells was detectable 2 to 4 h posttransfection and

FIG. 1. Electropherograms of immunoprecipitated VSV M, N, P, and G proteins present in the cytosol or released into the supernatant medium of CV1 cells transfected with recombinant pTM1 expression vectors. (A) Autoradiographs showing [³⁵S]methionine-labeled M, N, P, and G proteins synthesized in the cytosol or released from CV1 cells after incubation at 37°C for 8 h. (B) Time course of M-protein synthesis in the cytosol (C) and release into the supernatant (S) medium of M-gene recombinant pTM-O79-transfected CV1 cells incubated at 37°C for 2 to 16 h. Lanes VSV, marker M, N, P, and G proteins (A) or M protein alone (B) extracted from virions. G_s connotes the soluble, truncated, and hence faster-migrating G protein released from the cell surface (8). As described in the text, CV1 cells infected with T7 phage polymerase-expressing vTF1-6,2 and transfected with Lipofectin-assisted pTM1 recombinant vectors expressing G, N, P, or M proteins were incubated for 8 h (A) or 2 to 16 h (M protein) (B) in the presence of [³⁵S]methionine. Cell lysates or culture medium was collected, processed, immunoprecipitated, and autoradiographed after SDS-PAGE.

continued to accumulate in the cell culture medium until at least 16 h (Fig. 1B). No significant cell lysis was noted during incubation for 24 h. Similar results were obtained with transfected BHK-21 cells (data not shown), indicating exocytosis of VSV M protein (unaccompanied by other viral proteins) from intact mammalian cells. Only truncated G_s protein and no intact G protein could be found in the culture medium of cells transfected with the pTM-G gene recombinant, indicating different mechanisms for excretion of M and G proteins.

The presence of M protein in the culture medium of transfected cells did not appear to be due to detachment or lysis of cells, because no N, P, or intact G proteins were present in culture medium and no M protein was present in pelleted material after low-speed clarifying centrifugation. However, additional proof for cell viability and intactness of transfected-cell membranes was sought. Viability of CV1 cells transfected with M-gene-expressing pTM-OM79 was confirmed by finding that >97% of transfected cells were stained with the supravital dye neutral red; moreover, 30-min pulse-labeling with [³⁵S]methionine revealed that large amounts of ³⁵S-M protein and cellular proteins were still being synthesized at 16 h posttransfection (data not shown). This confirms earlier studies that VSV M protein alone does not cause cell death despite its marked capacity to induce cell rounding due to dissolution of cytoskeletal actin filaments (1, 20). In addition, we found that the plasma membrane of M-gene-transfected CV1 cells remains intact as determined by the exclusion of uptake of the dye trypan blue in >98% of 500 transfected cells, despite cell rounding (data not shown). These results clearly indicate that release of M protein from transfected cells is not due to cell

FIG. 2. Buoyant density (ρ) in the presence or absence of Triton X-100 of M protein present in the cytosol or released into the culture medium from cells transfected with M-gene recombinant pTM-OM79. Bar graphs represent the percentage of M protein in each of 10 fractions collected after isopycnic centrifugation of ³⁵S-labeled M protein released into culture medium (A), present in cytosol (B), released into culture medium to which Triton X-100 was added (C), or present in cytosol to which Triton X-100 was added (D). Autoradiographs after SDS-PAGE of M protein in each fraction are shown above the bar graphs. The percentage of M protein in each gradient fraction was determined by integrated scanning densitometry. CV1 cells were infected with vTF1-6,2 and transfected with the M-gene recombinant plasmid pTM-OM79, and the cell culture medium and cytosol were prepared 8 h after cells were labeled with [³⁵S]methionine. Clarified cell culture media and disrupted-cell cytosols were centrifuged at $150,000 \times g$ for 90 min, and the pelleted material was resuspended in 0.25 ml of PBS or $1 \times$ disruption buffer (20 mM Tris-HCl [pH 8.0], 5% glycerol, 3 mM dithiothreitol, 1% Triton X-100, 1 mM EDTA, 0.3 M NaCl) and layered over a 10 to 70% continuous sucrose gradient. The 10 to 70% sucrose gradients for the ³⁵S-M protein released into the cell medium or extracted from the cytosol were centrifuged at $100,000 \times g$ for 18 h, and 0.5-ml fractions were collected from the top of each gradient. Buoyant densities were measured with 200- μ l aliquots of each fraction. The remainder of each fraction was diluted to 1 ml in $1 \times$ NET gel buffer and immunoprecipitated with a monoclonal antibody specific for M protein. Each sample was analyzed by SDS-PAGE and autoradiography, and the amount of ³⁵S-M protein was quantitated by integrated densitometry.

lysis, leakiness, or entrapment of M protein in fragmented plasma membrane.

We next set out to determine whether the expressed VSV M protein was released from cells in association with plasma membrane fragments in the form of lipoprotein vesicles, as was demonstrated by electron microscopy of recombinant baculovirus-infected insect cells (9). Unfortunately, yields of M protein from mammalian cells were insufficient for electron microscopic demonstration of budded vesicles; moreover, vaccinia virus and Lipofectin rendered the plasma membrane of sectioned cells unsuitable for examination by electron microscopy (data not shown). Instead, to test for association with lipids, we compared the buoyant density of [³⁵S]methionine-labeled M protein present in the cytoplasm and released into the culture medium from CV1 cells transfected 8 h previously with the M-gene expression vector pTM-OM79 (Fig. 2). By

isopycnic sucrose (10 to 70%) gradient centrifugation, the mean buoyant density of M protein released from transfected CV1 cells was 1.12 g/cm^3 (Fig. 2A); that of the cytoplasmic M protein was 1.16 g/cm^3 (Fig. 2B). Exposure of the cell culture medium or cytosol of pTM-OM79-transfected cells to 0.5% Triton X-100 increased the buoyant density of both M proteins to 1.20 g/cm^3 (Fig. 2C and D).

The above experiment was repeated by colabeling pTM-OM79-transfected CV1 cells with [¹⁴C]leucine and [³H]palmitic acid before density gradient (10 to 70% sucrose) centrifugation and fractionation of radioactive M protein and lipid released into cell culture medium. The results of this experiment showed that ¹⁴C-M protein and ³H-lipid banded together at a buoyant density of 1.12 g/cm^3 , but the addition of Triton X-100 detergent converted the density of the ¹⁴C-M protein to 1.20 g/cm^3 (data not shown). The results of these two sets of experiments indicate that M protein released from pTM-OM79-transfected cells is associated with lipid, presumably as vesicles derived from plasma membrane, as shown previously by electron microscopy for M protein released from insect cells (9). No vesicles or lipid-containing components could be found in the media surrounding cells infected with vTF1-6,2 alone or cotransfected with plasmids expressing the N, P, or G gene (data not shown); these results indicate that vaccinia virus does not induce the release of vesicles from infected cells in the absence of M protein.

It was questioned whether vesicle formation and exocytosis of the VSV M protein are primarily functions of membrane-binding affinity and, if so, whether membrane-binding-deficient mutants of the M protein would be defective in promoting exocytosis. It has been shown that a certain proportion of intracellular M protein expressed during VSV infection or by vector transfection binds to the plasma membrane (4); truncated M protein lacking the amino-terminal basic domain still binds to plasma membrane but not as tightly (5). Therefore, it was of interest to compare the release of full-length and truncated M proteins from cells transfected with M-gene deletion mutants (16) pTM(M1-74) expressing only the first 74 amino acids and pTM(M51-229) expressing amino acids 51 to 229 and double-deletion mutant pTM(M51-74/107-229) expressing amino acids 51 to 74 and 107 to 229. Truncated M proteins expressed in CV1 cells by all three mutants, particularly those expressed by the double-deletion mutant, showed a reduced capacity to bind to the plasma membrane as determined both by fluorescent antibody staining and by cell fractionation (20). When full-length and truncated M proteins were expressed in CV1 cells, we found by autoradiographic integrated densitometry of SDS-polyacrylamide gel electrophoresis (PAGE)-separated [³⁵S]methionine-labeled M proteins that 31% of the full-length wt M protein was released into the culture medium from transfected CV1 cells; the CV1 cells transfected with pTM(M1-74) released 21% of the M protein truncated to the first 74 amino acids. By comparison, expression of pTM(M51-229) with deletion of the first 50 amino acids and pTM(M51-74/107-229) with deletion of amino acids 1 to 50 and 75 to 106 resulted in the release into the culture medium of only 2.4 and 6.8%, respectively, of these truncated M proteins (data not shown). It seems likely from these observations that the region of the M protein containing the first 50 N-terminal amino acids, which also encompasses one of the membrane-binding sites, is essential for efficient exocytosis of the VSV M protein. Since the truncated protein M1-74 does not cause cytopathic rounding of CV1 cells (20), this experiment also provides additional evidence that exocytosis of M protein is not due to a cytopathic effect.

Clearly, the capacity of the VSV M protein to induce the

FIG. 3. SDS-PAGE autoradiographs (A) and schematic stick model (B) of M proteins present in the cytosol (C) and released into the medium supernatant (S) from CV1 cells transfected with pTM1 recombinants of the wt, site-specific mutants, *ts*O23, and site-specific *ts*O23 revertants of the VSV M gene at the permissive (31°C) and nonpermissive (39°C) temperatures. (A) Shown are the amino acids (a.a.) at residues 21, 111, and 227 of M proteins expressed by M genes of the wt, three site-specific mutants (M1, M2, and M3), the temperature-sensitive mutant *ts*O23, and three site-specific revertants (R1, R2, and R3). Autoradiographs of ³⁵S-M protein are shown for cytoplasmic extracts (C) and cell medium supernatants (S). The lanes designated VSV show the marker M protein extracted from virions. (B) Schematic model of M proteins showing the positions of amino acid transitions in wt and *ts*O23 M proteins. Mutants M1, M2, and M3 of the wt M gene and revertants R1, R2, and R3 of the *ts*O23 M gene were originally made in this laboratory by single-nucleotide exchanges leading to amino acid substitutions at the positions indicated (10). These mutants and revertants were then subcloned into the pTM1 expression vector. CV1 cells were transfected by these plasmids, and after incubation for 8 h at the permissive (31°C) or nonpermissive (39°C) temperature in the presence of [³⁵S]methionine, cytoplasmic extracts and cell culture media were processed as described in the legend to Fig. 2. Immunoprecipitated M proteins in the cytosol and those released into the cell culture medium were subjected to electrophoresis on 12.5% polyacrylamide-SDS gels and autoradiographed.

formation of vesicles that bud from the plasma membrane is dependent on its membrane-binding affinity. In all likelihood the structural conformation of the M protein must also play a key role in membrane budding and exocytosis. We had previously also found that the M protein synthesized in cells infected with the M gene of temperature-sensitive mutant *ts*O23 failed to bind to plasma membrane at the restrictive temperature of 39°C but did so at the permissive temperature of 31°C (20). The M gene of *ts*O23 has three mutations leading to amino acid substitutions at residues 21, 111, and 227; the temperature-sensitivity restriction phenotype has been shown to be due to a Leu→Phe substitution at amino acid 111 (11). Plasmid expression of M protein with leucine at residue 111 will complement *ts*O23 infection by enhancing the release of virions at the restrictive temperature, but the presence of phenylalanine or other amino acids at residue 111 results in a failure to complement *ts*O23 infection (10).

In order to determine whether exocytosis of M protein was influenced by mutations at any of the three sites in the *ts*O23 M gene, site-directed mutants of the wt M gene and revertants of the *ts*O23 M gene constructed for earlier studies (10) were subcloned into pTM1 (Fig. 3). A schematic model (Fig. 3B) shows the locations of the three different amino acids in wt and *ts*O23 M proteins in order to illustrate the site of each single mutation in the wt or each revertant in the *ts*O23 M proteins. Figure 3A also lists the amino acids at residues 21, 111, and 227 for the wt M protein and each site-specific mutant (M1, M2, and M3) as well as the *ts*O23 M protein and its site-specific revertants (R1, R2, and R3). Each mutant and revertant expressed about the same amount of M protein (varying not

TABLE 1. Comparative release of M protein^a

M-gene vector	Amino acid change(s)	% M protein released	
		31°C	39°C
pTM-OM79	wt	41	35
pTM-OM79(M1)	Gly-21→Glu	35	29
pTM-OM79(M2)	Leu-111→Phe	19	<1
pTM-OM79(M3)	His-227→Tyr	44	24
pTM- <i>ts</i> O23	Glu-21, Phe-111, Tyr-227	22	<1
pTM- <i>ts</i> O23(R1)	Glu-21→Gly	16	<1
pTM- <i>ts</i> O23(R2)	Phe-111→Leu	39	34
pTM- <i>ts</i> O23(R3)	Tyr-227→His	20	<1

^a Data were obtained from Fig. 3 by integrated densitometry of SDS-PAGE autoradiographs. Shown is a comparison of the release of M proteins at the permissive (31°C) and nonpermissive (39°C) temperatures from CV1 cells transfected 8 h previously with wt, mutant (M1, M2, and M3), *ts*O23, and revertant (R1, R2, and R3) M-gene recombinants.

more than twofold by densitometry) in the cytoplasm (Fig. 3A, lanes C) of transfected cells incubated at the permissive (31°C) or nonpermissive (39°C) temperature. However, cells transfected with certain constructs (expressing M proteins M2, *ts*, R1, and R3) and incubated at 31°C released somewhat smaller amounts of M protein (about 25% of that of the wt) into the culture medium supernatant (Fig. 3A, lanes S). The common feature of all four of these M-gene clones is a phenylalanine at residue 111, suggesting some restriction of M-protein exocytosis even at the permissive temperature of 31°C (Fig. 3A, lanes 6, 10, 12, and 16).

Table 1 summarizes the data in Fig. 3 by comparing integrated densitometry of M proteins released at 31 and 39°C. Cells transfected with the wt M-gene clone (Gly-21, Leu-111, and His-227) released about the same amount of M protein into the culture medium after incubation at 31 or 39°C (lane 2), whereas the *ts*O23 mutant M-gene clone (Glu-21, Phe-111, and Tyr-227) released no detectable M protein into the culture medium when incubated at 39°C (Fig. 3A, lane 10). The retardation in electrophoretic migration of the expressed *ts*O23 M protein is due to the Gly→Glu substitution at amino acid 21 (10). The most striking finding was in the expression of mutant M2 (Leu-111→Phe), in which no M protein could be detected in the culture medium after incubation at 39°C despite ample release into the culture medium at 31°C (Fig. 3A, lane 6); comparable amounts of M protein were present in the cytoplasm at both 31 and 39°C (Fig. 3A, lane 5). In sharp contrast, wt M-gene site-directed mutants M1 (Gly-21→Glu) and M3 (His-227→Tyr) had no significant effect on the release of M protein from cells transfected at 39°C (Fig. 3A, lanes 4 and 8). Conversely, the revertant R2 in the *ts*O23 M gene (Phe-111→Leu) was restored in its capacity to release M protein into the culture medium of CV1 cells transfected at 39°C (lane 14), whereas cells transfected with *ts*O23 M-gene revertants R1 (Glu-21→Gly) and R3 (Tyr-227→His) remained completely devoid of any capacity to release M protein from transfected cells incubated at 39°C (Fig. 3A, lanes 12 and 16).

Concerted efforts were made to determine whether the *ts*O23 virus itself was capable of inducing exocytosis of mutant M protein. Only leaky virions containing gel-retarded mutant M protein could be found in the culture medium of [³⁵S]methionine-labeled CV1 cells infected with *ts*O23; no free M protein or vesicles containing M protein were released from cells infected at 31 or 39°C with *ts*O23 over a period of 18 h (data not shown). These results differ somewhat from the data of Schnitzer and Lodish (14), who reported the release of

subviral particles from Vero cells infected at the nonpermissive temperature with other M-gene VSV temperature-sensitive mutants, *tsG31*, *tsG32*, *tsG33*, and three others isolated at the Massachusetts Institute of Technology but thought to be derived from the Glasgow *tsG33* mutant. They found, by rate zonal and buoyant density centrifugation, noninfectious viral particles deficient in N protein and RNA released from cells infected with *tsM301*; these released subviral particles contained normal amounts of G protein compared with the G protein-free particles released from cells at 39.5°C after being infected with the G-gene mutant *tsO45* (14). These noninfectious virions released from cells infected at the nonpermissive temperature were not further characterized, and no studies were performed with the *tsO23* M-gene mutant studied by us. Schnitzer and Lodish concluded from their pioneering studies published in 1979 (14) that the M protein is the “key for VSV bud formation.” Our quite different findings are nonetheless consistent with their conclusion.

Evidence that the matrix protein of VSV, in the absence of other viral proteins, induces the formation of lipoprotein vesicles which bud from the external surface of the plasma membrane of mammalian cells is presented here. Among the numerous putative functions of the VSV M protein are the capacities to condense ribonucleoprotein cores (13), to down-regulate viral transcription (3), to induce cell rounding (1), to interact with the plasma membrane (4), and to promote budding of progeny virions (18). We have recently identified by deletion mutation two sites on the VSV M protein responsible for membrane binding *in vivo* and *in vitro* (20); one of these membrane-binding sites within the first 50 N-terminal amino acids was found to be essential for inducing exocytosis. Also recently reported in the same paper (20) was the finding that the M protein of the *tsO23* mutant was restricted in its capacity to bind the plasma membrane of cells infected at the nonpermissive temperature. Previous studies had also shown that phenylalanine at residue 111 in the M protein is responsible for the restricted production at 39°C of *tsO23* virions (11) which could be rescued by complementation with M protein containing leucine at residue 111 (10). These past and present data indicate that a specific structural conformation of the M protein is responsible for the formation and budding of vesicles from the plasma membrane, a property of the M protein which probably also promotes VSV assembly and budding of virions from the host cell. This property of membrane binding and vesicle formation of M protein is apparently independent of other viral components and implicates the M protein as the sole factor that promotes the budding of VSV virions.

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